PATHOGENETIC ROLE OF MESANGIAL IMMUNE DEPOSITS

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Summary

This investigation was designed to clarify the pathogenetic role of immune complexes within the mesangium. We administered a ferritin-protein antigen to rats which localised and persisted within the mesangium. Subsequently administration of anti-ferritin antibody resulted in binding of antibody to the mesangially located antigen. The effect of this immune complex formed in situ was studied in long-term experiments and no evidence of glomerular impairment was seen. These results lend support to the view that an immunological process, confined to the mesangium, may not necessarily compromise renal function.

Introduction

The deposition of immunoglobulins in a mesangial pattern is a common finding in many cases of glomerulonephritis [1]. The exact nature of these deposits is unknown, although they are widely believed to represent immune complexes. The prognosis of glomerulonephritis where immune deposits are confined to the mesangium is generally good, but progression to renal failure can occur [1]. Previous investigations, using different animal models which parallel the immune pathology of the human disease, have produced contradictory results as to the effect of mesangially located immune complexes on the glomerulus [2–5]. We have previously described a system [6] whereby a ferritin-protein conjugate is introduced into the mesangium of rats following intravenous administration. The material is rapidly removed from the circulation, but persists for extended periods within the mesangium. The subsequent injection of anti-ferritin antibody results in the formation of immune complexes in situ. We here present the long-term observations on renal function using this model.

Material and Methods

Experiments were carried out with male Wistar strain rats of 100g body weight. The ferritin-HSA or-IgG conjugates and rabbit anti-ferritin antiserum were pre-
pared as previously described [6]. In addition anti-ferritin anti-serum was raised in Wistar rats by immunising them with 1mg of ferritin in FCA intramuscularly twice at a 4-week interval. The rats were bled out after 6 weeks. Fresh guinea pig serum was used as a source of complement. The experimental design was as shown in Table I.

<table>
<thead>
<tr>
<th>Antigen (per 100g)</th>
<th>Interval</th>
<th>Antiserum i.v. (per 100g)</th>
<th>Group*</th>
</tr>
</thead>
<tbody>
<tr>
<td>50mg Fe-IgG</td>
<td>3 days</td>
<td>1ml rabbit anti-Fe</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1ml normal rabbit serum</td>
<td>2</td>
</tr>
<tr>
<td>50mg Fe-IgG</td>
<td>15 days</td>
<td>1ml rabbit anti-Fe</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1ml normal rabbit serum</td>
<td>4</td>
</tr>
<tr>
<td>50mg Fe-HSA</td>
<td>3 days</td>
<td>1ml rat anti-Fe</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1ml normal rat serum</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1ml rabbit anti-Fe</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>8</td>
</tr>
</tbody>
</table>

*Groups 1–6 contained 20 rats each. Groups 7–8 contained 10 rats each.

One day after antigen administration and 2hr, 24hr, 7 days, 3 months, 11 months and 18 months after antibody injection 2 rats from groups 1–6 inclusive were sacrificed and samples of renal tissue were taken. Rats from groups 7 and 8 were examined at 7 days, 3 months and 11 months respectively. Cryostat sections were stained with FITC or TRITC labelled antisera to ferritin, rabbit Ig, rat Ig and rat C3 and grading of the sections was performed as described previously [6]. Paraffin sections were examined by light microscopy without prior knowledge of the treatment. Urine samples were collected immediately following antigen and antibody administration, then at weekly intervals and from three months onwards at monthly intervals. Fresh urine was examined for the presence of red and white cells and screened for protein with a TCA precipitation test.

Results

Following intravenous injections of the ferritin-HSA and IgG conjugates, intense fluorescence for ferritin was seen in a mesangial pattern (Figure 1a). This antigen was accessible to specific rabbit or rat anti-ferritin antibody given 3 or 15 days afterwards, as shown by immunofluorescence (Figure 1b).

After reaction of the antibody with the mesangially-deposited antigen, staining for rat C3 was more intense in the animals receiving rat anti-ferritin, but
was still faint. In an attempt to increase the binding of complement, in preliminary experiments rats were given 1ml of fresh guinea pig serum 30min after the antibody. Very little additional binding of guinea pig C3 was seen and in all subsequent experiments it was decided to inject anti-ferritin alone.

The passively administered antibody could be detected in the circulation at 2hr, by a gel diffusion test, but was absent or very weak by 24hr. In groups 1–6 the intensity of staining for ferritin within the mesangium declined slowly with time but faint staining was still visible in some animals at 18 months. There were no systematic differences between the groups. Immunofluorescent staining for rabbit Ig, rat Ig and rat C3 was carried out up to and including 3 months. In groups 1 and 3 intense staining for rabbit Ig was seen at all times, whereas staining for rat Ig was much weaker although fairly constant. In groups 2 and 4 staining for rat Ig was marked between days 7 and 14, decreasing thereafter. In group 5 staining for rat Ig was always strong. Deposition of rat C3 was never impressive in any group. Urinary abnormalities could not be detected either immediately following antigen or antibody administration, nor at any time thereafter; a slight rise in total urinary protein excretion was paralleled in untreated controls and presumably reflected the effect of ageing. Morphologically, after antigen administration but before the antibody, an increase in the numbers of mesangial and endothelial cells was seen. The cells appeared swollen with enlarged and segmented nuclei. These changes were similar in degree in groups 1–6, and initially increased with time (whether or not antibody had been given), being most pronounced between the first and fourth weeks after serum administration (Figures 2 and 3).

After 3 months all glomeruli examined were largely normal and no further changes were seen (Figure 4).

Polymorph infiltration was in general not conspicuous although some animals in group 2 had more marked infiltration early on. No noteworthy changes were seen in the control groups 7 and 8.
Figure 2. Glomerulus 24hr after injection of rabbit anti-ferritin antibody to a rat given ferritin-IgG conjugate 3 days previously. Note areas of proliferation (arrowed) (PAS X 410 – reduced for publication)

Figure 3. Glomerulus 24hr after injection of normal rabbit serum (control) to a rat given ferritin-IgG conjugate 3 days previously. Proliferation arrowed. (PAS X 410 – reduced for publication)
Figure 4. Glomerulus 3 months after injection of normal rabbit serum (control) to a rat given ferritin-IgG conjugate 3 days previously. Largely normal (PAS X 380 – reduced for publication)

Discussion

The role of the mesangium as a primary target in glomerular disease has been investigated in several experimental systems. In the model described here we selected conditions under which mesangial uptake of a protein complex is maximised. Viewing this as a ‘planted antigen’ we then established a mesangially localised in situ immune complex by subsequent passive administration of antibody. Since this antibody still circulated several hours later it was apparent that we had saturated the available antigenic determinants. In spite of the impressive quantities of complex thus introduced we failed to detect any alteration in glomerular function, as judged by increased permeability of the GBM. We did see morphological changes, mainly an increase in the numbers of mesangially located cells; but this occurred in all groups receiving antigen, regardless of whether specific antibody was given or not. Hence the morphological changes seem to be an antigen-induced reaction and not directly related to the immune complex.

A similar model was described first by Mauer and co-workers [2,7] using aggregated HuIgG as antigen. They reported the development of acute glomerular injury in rabbits and rats. This was based solely on morphological criteria and no functional parameters were measured, but the changes seen were much more marked than in our experiments. In our opinion the fact that these workers administered the specific antibody after renal transplantation may be important, since it could be that a transplanted kidney reacts more readily to
an immunological insult. Two other reported systems have involved chronic immunisation schedules with high molecular weight antigens, leading to mesangial immune complex deposition. Ferritin was employed by Stilman et al [4] and they induced glomerulonephritis with marked proteinuria in rats, the type of lesion being of the 'mixed' form and the GBM was affected. Germuth and co-workers [5] gave thyroglobulin to rabbits and found varying degrees of mesangial cell proliferation and matrix increase, but rarely any urinary abnormalities. Proteinuria occurred only in animals with additional GBM involvement. This latter model has the drawback of heavy loss of animals from anaphylaxis and thyrotoxicosis. A further report [3] describes the development of glomerulonephritis in dogs following adenovirus infection. Proteinuria was seen, but again this lesion appears to be a mixed form. A limitation is the high mortality seen after 1 to 2 weeks. There are other reports dealing with the effect of immune processes in the mesangium, but the pathogenetic mechanisms were either clearly different from those discussed above (anti-mesangium antibody [8,9]) or unknown [10].

Our data show that the presence of readily detectable quantities of immune complexes within the mesangium does not necessarily compromise renal function. It is tempting to speculate that when a certain level of mesangial immune complex deposition is exceeded the process 'spills over' onto the GBM and only at this point does clinical disease appear.

We compared the effect of giving anti-ferritin antibody from the rabbit or the rat. In the former case, by analogy with anti-GBM nephritis, one might expect an autologous phase due to a host reaction to the planted rabbit IgG. However little fixation of rat IgG occurred, even after 3 months in these animals. It seems that rabbit antibody is of no advantage and we now prefer to use rat antibody to give a 'cleaner model'.

The persistence of ferritin-protein conjugates has been previously reported [6]; in the current work it has been shown that antigenic activity can be detected up to 18 months after administration. Interestingly the subsequent fixation of antibody does not seem to markedly influence persistence.

References


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Open Discussion

KOENE (Nijmegen) Is it possible that the mesangial immune complexes do not harm the kidney because there is no 'fixed antigen' involved? It has been suggested that in epimembranous nephropathy fixed antigen might play an important pathogenetic role.

BATSFORD That is a good point. I don't really see why there should be any difference between an immune complex consisting of fixed antigen plus antibody, and one which consists of antigen which has been introduced into the mesangium plus antibody, except when the exact locus is critical. I mean if the fixed antigen is in a particular site which is not accessible to an antigen which is introduced from the circulation and this site is crucial, then there could be a difference, otherwise I cannot see why there should be a difference in the effect.